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The Effect of AlloDerm on the Initiation and Growth of Human Neovessels

Sean R. Weiss, MD; Justin M. Tenney, MD; Jessica L. Thomson, PhD; Catherine T. Anthony, PhD; Ernest S. Chiu, MD; Paul L. Friedlander, MD; Eugene A. Woltering, MD

Objectives/Hypothesis: AlloDerm (LifeCell Corp., Branchburg, NJ) is commonly employed for reconstruction of ablative soft tissue and mucosal defects following surgical resections. Although devoid of growth factors, AlloDerm may serve as an adhesive matrix for binding of growth factors, increasing local angiogenesis, and wound healing. We hypothesized that AlloDerm would enhance angiogenesis and might be altered with autologous blood products to enhance initiation of the angiogenic response.

Methods: We used a human placental vein in a fibrin-thrombin clot-based angiogenesis model. Four groups, human placental vein (HPVM), HPVM with AlloDerm, HPVM with AlloDerm plus platelet-poor plasma, and HPVM with AlloDerm plus platelet-rich plasma were evaluated. Endothelial cell growth was evaluated visually (40×). Hematoxylin and eosin staining and immunofluorescent staining for growth within the AlloDerm matrix were also performed. To assess human umbilical vein endothelial cell (HUVEC) sites of attachment to AlloDerm, we incubated HUVEC cells with AlloDerm for a period of 2 weeks and evaluated attachment with anti-factor VIII immunofluorescence.

Results: Angiogenic initiation decreased in the combined placental vein with AlloDerm group ($P < .0001$ at day 7, 14, 21). Additionally, initiation in the AlloDerm plus platelet-poor plasma group was significantly better than the AlloDerm alone group when placentas 2 and 3 were compared ($P < .0001$). On hematoxylin and eosin staining and immunofluorescent factor VIII staining, no endothelial growth into the AlloDerm was noted in the samples analyzed.

Conclusions: AlloDerm may be enriched with platelet-poor plasma to stimulate greater initiation and wound healing; however, AlloDerm inhibits angiogenic initiation in this model.

Key Words: AlloDerm, angiogenesis, wound healing, skin.

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INTRODUCTION

Acellular allogenic dermis has been used for the reconstruction of a variety of postoperative head and neck defects, including parotid ablative defects, tongue, floor of mouth, skull base, and pharyngeal reconstructions.[1–6] It has also been extensively studied and felt to be effective in preventing Frey's syndrome following parotidectomy.[7] AlloDerm (LifeCell Corp., Branchburg, NJ) has been noted to have a role in improving long-term cosmesis when used in combination with fat injections.[8] This collagen-based product is immunologically inert, has good integrity, and is readily incorporated into host tissues. AlloDerm is superior to autografting in decreasing donor site morbidity and is readily available. Rigorous detergent exposure during production of AlloDerm renders it essentially devoid of growth factors or immune reactive cells; however, an extracellular matrix (ECM) of type I and type III collagen, along with proteoglycans and glycosaminoglycans remains intact. Proteoglycans and glycosaminoglycans, along with fibronectin, may serve as binding sites for growth factors and endothelial cells, respectively, thereby promoting angiogenesis.

During wound healing, angiogenesis primarily occurs from the cut endothelial surface of pre-existing vessels. Proteolytic degradation of the basement membrane of the parent vessel by metalloproteinases and disruption of cell-to-cell contact by plasminogen activator precedes migration of endothelial cells toward an angiogenic stimulus. The provisional ECM, composed of fibronectin, plays a critical role in the process of wound healing through binding to ECM proteins.[9] Integrins are critical for the formation of new blood vessels through binding to ECM components such as fibronectin,

From the Department of Otorhinolaryngology–Head and Neck Surgery (S.R.W., J.M.T.) and the Department of Surgery (C.T.A., E.S.C., A.E.W.), Louisiana State University Health Sciences Center, New Orleans, the U. S. Department of Agriculture, Agricultural Research Service, Southern Regional Research Center, Baton Rouge (J.L.T.), and the Department of Otolaryngology, Tulane University Health Sciences Center, New Orleans (P.L.F.), Louisiana, U.S.A.

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Send correspondence to Justin M. Tenney, MD, Louisiana State University Health Sciences Center, Department of Otolaryngology–Head and Neck Surgery, 533 Bolivar Street, New Orleans, LA 70112.
E-mail: jtenne@lsuhsc.edu

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regulating the activity of vascular endothelial growth factor receptor-2, and interacting with matrix metalloproteinase-2. Proteinases cleave ECM proteins, releasing growth factors that stimulate angiogenesis. Dissolution of the ECM allows migration of endothelial cells at the initiation of angiogenesis.

Histological components of the AlloDerm matrix include mature elastin, proteoglycans, fibronectin, and collagen. Naturally occurring ECM proteoglycans provide a reservoir for growth factors and are directly involved in angiogenesis. Thus, on a molecular level, allogenic acellular dermis provides a structural proteoglycan matrix that can potentially serve as an adhesive reservoir for growth factors.

Platelet-rich plasma is a hemostatic autologous blood product with implications in wound healing. These platelet-rich plasma products have increased concentrations of growth factors, platelets, and white blood cells that have previously been verified quantitatively.[10] Platelet-poor plasma, Vitagel (Orthovita Corp., Malvern, PA), contains growth factors, a lesser quantity of platelets, and fibrinogen, which in the presence of collagen form a collagen/fibrin gel matrix for hemostasis. Its potential for use in the clinical setting as a hemostatic agent and expeditor of wound healing has been previously described.[10–12] Delivery of these products locally in a wound bed might stimulate angiogenesis and enhance wound healing.

A reproducible human placental vein angiogenesis assay can be used to simulate a healing wound.[13–16] The angiogenic stimulating potential for AlloDerm alone, and in combination with autologous blood products, can be evaluated using this model. We hypothesized that the presence of AlloDerm in our assay would stimulate the initiation of neovessel sprouting and would increase subsequent proliferation of neovessels. We also anticipated that addition of autologous blood products to the AlloDerm would stimulate the initiation of neovessel sprouting and would increase subsequent growth. Based on the results of these experiments, we could determine if AlloDerm provides an environment conducive to angiogenesis or simply acts as a volume occupying allograft.

MATERIALS AND METHODS

Tissue Preparation

To test these hypotheses, human placentas were obtained at the time of delivery with the approval of the Louisiana State University Health Sciences Center Institutional Review Board (New Orleans, LA). Placentas were topically cleansed with chloramine T (1 g/L), and surface veins were identified and dissected free of their investing tissue. Excess adventitial tissue was removed, and veins were cut along their long axis to create a sheet of full-thickness vein tissue. A sterile 2 mm skin punch was used to create discs of placental vein tissue. Tissue samples were allocated into wells of a 96-well plate (Corning Inc., Corning, NY) in a random manner. Each well had been were pretreated with thrombin solution (0.05 IU in 1.0 μL/well) (Sigma Chemical Co., St. Louis, MO), and allowed to dry before use. For each specimen, 30 to 60 wells were prepared for controls and for each experimental group. Fibrin-thrombin clots were created within each well using previously described methods.[14–17] Briefly, following placement of tissue in the bottom of thrombin-containing wells, the fragments were covered with 100 μL of a clot-forming medium consisting of human fibrinogen (3 mg/mL) and ε-amino caproic acid (0.5%) (Sigma) in HPVAM medium. HPVAM medium contained Medium 199 and an antibiotic/antimycotic solution consisting of 100 U penicillin, 100 U streptomycin sulfate, and 0.25 μg amphotericin/mL (Gibco BRL, Gaithersburg, MD). This mixture was placed in a humidified incubator and allowed to clot at 37°C in a 6% CO_2, 94% air environment. A nutrient medium (100 μL) containing the HPVAM supplemented with 20% bovine serum (Gibco BRL) was added to the tissue-containing clot. The total volume was 200 μL per well.

AlloDerm Information

The AlloDerm was rehydrated according to manufacturer's instructions. Subsequently, a sterile skin punch was used to create 2-mm-diameter discs of AlloDerm. In placenta 1 (n = 60), AlloDerm ranging in thickness from .79 to 1.02 mm in thickness was utilized. In placentas 2 and 3 (n = 60), AlloDerm of thickness .15 to .30 mm was used.

Preparation of Platelet-Rich Plasma

Platelet-rich plasma was prepared in our laboratory according to the protocol outlined below using the Gravitational Platelet Separation System (Cell Factor Technologies, Biomet, Warsaw, IN). Briefly, 60 mL of blood were drawn from study patient 1 and centrifuged for 12 minutes at 3,200 rpm. After centrifugation, the buffy coat layer with concentrated platelets and white blood cells was removed. This yielded 5.5 mL of platelet-rich plasma. A thrombin (1,000 IU/mL) and CaCL2 (100 mg/mL) solution was formulated in a separate syringe to yield a total volume of 5.5 ml (0.5 mL thrombin in 5 mL CaCl2).

Preparation of Vitagel

Vitagel is formulated using platelet-poor plasma. In this system 12 mL of blood are centrifuged, yielding a 4-cc volume of coagulation factors, fibrinogen, growth factors, and a 2× to 3× concentrate of platelets. This centrifugation product is then combined in a sprayable form with a 4-mL volume of thrombin (300 U/mL), collagen (20 mg/mL), and calcium chloride (40 mM) admixture.

Preparation of Human Umbilical Vein Endothelial Cells

To determine sites of attachment of endothelial cells to AlloDerm, we incubated 4-mm punches of AlloDerm either with basement or dermal side up with a concentration of 28,000 human umbilical vein endothelial cells (HUVEC)/mL. These Alloderm-HUVEC discs were incubated at 37°C in 6% CO_2, 94% air and allowed to grow for 7 days until confluent. Media was changed at days 7, 11, 14, and 18.

Treatments

Human placental vein discs were plated alone, with AlloDerm, with AlloDerm plus platelet-poor plasma, and with AlloDerm plus platelet-rich plasma. When AlloDerm was added to the HPVAM model outlined above, it was placed at the bottom of the well prior to the addition of the placental vein disc. The AlloDerm was oriented with the dermal side facing up, and the placental vein disc was placed directly on top of the AlloDerm discs. The plated tissue was then embedded in a fibrin-

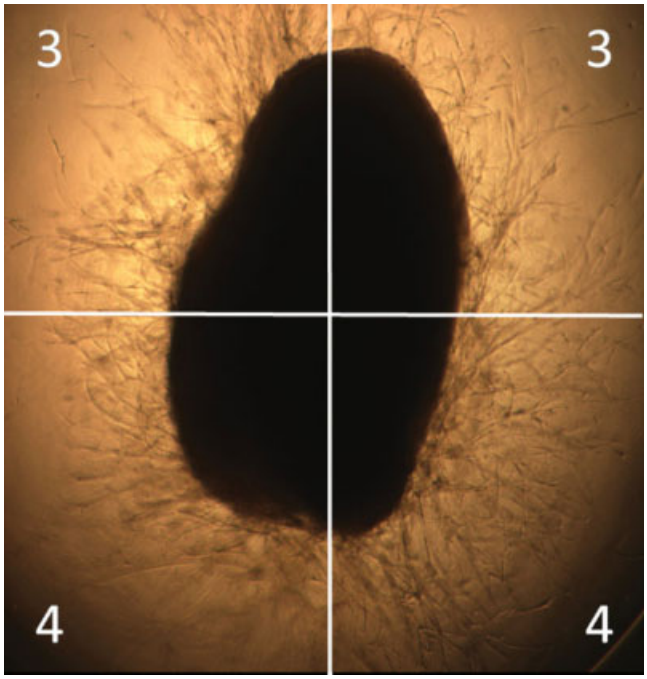

Fig. 1. Placental vein disc undergoing angiogenesis. Discs are divided into quadrants and each quadrant is given a score of 1 to 4. A sum of the 4 quadrants is then obtained for a potential total score of 16. This example's score is 3 + 3 + 4 + 4 = 14. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

thrombin clot and treated with nutrient media according to the HPVAM protocol listed above. When platelet-rich plasma or platelet-poor plasma was used in conjunction with AlloDerm, it was used to coat the AlloDerm discs prior to placing them in the wells. Media was changed at days 7 and 14.

Evaluation of Angiogenesis

Wells were assessed over time using an inverted phase microscope (20×–40×). The observers had no bias as to the effect any of the treatments would have on angiogenesis, and two-person scoring was performed for validation. Two different assay criteria were evaluated: 1) initiation of sprouting (% initiation [%I]), and 2) length, density, and percent of circumference (angiogenic index [AI]). Initiation of an angiogenic response was defined as the development of three or more spouts around the periphery of the tissue (%I; number of wells angiogenic/total prepared × 100). This measurement reflects the effect of the treatments on the earliest phase of angiogenesis in the vein model. The subsequent growth and development of an angiogenic response (the AI) was assessed using a semiquantitative visual rating scale. Briefly, discs were visually divided into four quadrants. Each quadrant was given a numeric score from 0 to 4 based on neovessel length, density, and percentage of the quadrant's circumference involved with the angiogenic response. Numeric results from the four quadrants were summed and expressed as a semiquantitative angiogenic index (AI, 0–16) (Fig. 1). These scores correlate well with more objective measures of vessel growth, such as vessel length or vessel surface area, as determined by digital image analysis.[15] Angiogenic index scores between multiple trained observers have a high degree of correlation. Angiogenic scores for wells from the treatment groups or the controls were averaged and presented in two formats: 1) either excluding scores from wells with no growth (AI without zeros), or 2) including scores from all wells including wells with no growth (AI with zeros). The AI with zeros excluded group represents neovessel growth, because only those wells exhibiting an angiogenic response were included in the analyses. In contrast, the AI with zeros included group reflects the combined effects of the first two measurements, because it takes into account the AI of all wells, whether or not they produced a visible angiogenic response. To further qualitatively assess endothelial growth into the fibrin thrombin clot and AlloDerm, selected samples from each group were chosen for hematoxylin and eosin and factor VIII staining.

Statistics

Three different measurements were of interest, each requiring different statistical analyses. The first measurement of interest was the proportion of wells that initiated an angiogenic response (%I). This measurement reflects the effect of the treatments on the earliest phase of angiogenesis. The second measurement of interest was the AI. This value was treated as a continuous measurement even though it is similar to a count-type measurement. This measurement reflects the effect of the treatments on the growth of neovessels, because only those wells that exhibited an angiogenic response were included in this analysis. These analyses are referred to as AI without zeros. The third measurement of interest is also called the AI, but uses all of the wells plated, including the wells that did not initiate an angiogenic response (zero values). Hence, these analyses are referred to as AI with zeros. This third measurement reflects the combined effects of the first two measurements, because it takes into account the AI of all wells, whether or not they initiated an angiogenic response. Results were considered significant at the nominal level of 0.05 unless otherwise noted.

Initiation among the four treatment groups was compared using a χ^2 test of association for each of the 3 measurement days. If the overall table value was significant, then pair-wise comparisons of the treatment groups were performed using a χ^2 test of association run with a Bonferroni correction factor applied to the nominal level to maintain an overall type I error rate of 0.05. The individual pair-wise significance level was set at 0.0033 (15 comparisons).

Analysis for the AI required the use of a mixed-models approach using an unstructured covariance matrix that was allowed to vary in the treatment groups (i.e., account for heterogeneity between groups). The mixed-models approach was used to account for the longitudinal component (multiple days of measurement) of the study. Since the focus of the study was to determine if the treatments differed from one another in each of the 3 days, post hoc F tests were conducted to test for treatment differences within each day. If the F test was significant, then Tukey-Kramer adjusted P values for pair-wise comparisons among treatment groups within the 3 days were computed based on least-squares mean estimates.

RESULTS

We compared the angiogenic effects of the various treatment combinations; AlloDerm alone, AlloDerm in platelet-poor plasma, and AlloDerm in platelet-rich plasma. The percent initiation and AI with and without zero values were determined in the treatment wells derived from the three placental veins. In placental vein 1, only the AlloDerm alone was evaluated. In placental veins 2 and 3, all three treatment combinations were evaluated. However, for both these placental veins, the

TABLE I.
Initiation.

Treatment Group	% Initiation	Comparison *P* Value		
		AlloDerm	AlloDerm + PPP	AlloDerm + PRP
Days in culture = 7				
Control	45.4	<.0001	0.0020	<0.0001
AlloDerm	10.0		0.0329*	0.2526
AlloDerm + PPP	21.7			0.0072*
AlloDerm + PRP	5.0			
Days in culture = 14				
Control	82.4	<.0001	0.0049*	<.0001
AlloDerm	50.0		0.0904	<.0001
AlloDerm + PPP	63.3			<.0001
AlloDerm + PRP	18.3			
Days in culture = 21				
Control	84.0	<.0001	0.0079*	
AlloDerm	52.5		0.0701	
AlloDerm + PPP	66.7			

*Not significant at Bonferroni-corrected individual nominal level = 0.0033.
PPP = platelet-poor plasma; PRP = platelet-rich plasma.

AlloDerm in platelet-rich plasma treatment group became contaminated between day 14 and 21 in culture. Thus, no data is shown for day 21 in culture for this treatment group.

Initiation

Initiation in control wells (percent of wells initiating an angiogenic response divided by the total number of wells plated) was compared to initiation in treated wells for the different combinations of AlloDerm (Table I). For all 3 days, overall χ^2 tests of association revealed significant differences among the four treatment groups. Subsequent pair-wise comparisons of the treatment groups using χ^2 tests of association were then conducted revealing the following results. On day 7, all three treatment groups, AlloDerm alone, AlloDerm in platelet-poor plasma, and AlloDerm in platelet-rich plasma exhibited significantly less initiation than did the control group ($P < .0001$, $P < .0020$, and $P < .0001$, respectively). On day 14, only the AlloDerm alone and AlloDerm in platelet-rich plasma groups exhibited significantly less initiation than did the control group ($P < .0001$). There was no significant difference in initiation between the AlloDerm in the platelet-poor plasma group and the control group. In addition, the AlloDerm in platelet-rich plasma exhibited significantly less initiation than did either the AlloDerm alone or AlloDerm in platelet-poor plasma groups. There was no significant difference in initiation between the AlloDerm alone and AlloDerm in platelet-poor plasma groups. On day 21, only the AlloDerm alone treatment group exhibited significantly less initiation than the control group. There were no significant differences between the AlloDerm in the platelet-poor plasma group and the control group, or between the AlloDerm and AlloDerm in platelet-poor plasma groups (Fig. 2).

Angiogenic Index Without Zeros

This analysis was performed only for those treatment groups that exhibited an angiogenic response (wells with a score of 0 were excluded from analysis). The analysis using a mixed-models approach revealed significant tissue (placental vein), day (number of days in culture), treatment and interaction (day × treatment) effects ($P < .0001$). The tissue effect was most likely caused by the AlloDerm alone treatment being applied to placental vein 1 and not the other two AlloDerm combinations, as was done in placental veins 2 and 3. The day effect simply verifies that angiogenesis increased over time. Because the day × treatment interaction effect was significant, post hoc F tests were conducted to check for significant treatment effects within each of the 3 measurement days. Results of the F tests revealed significant treatment effects for all 3 days, 7, 14, and 21 ($P = .0089$, $P < .0001$, and $P < .0001$, respectively). Based on Tukey-Kramer adjusted P values for pair-wise comparisons among treatment groups within the 3 days (Table II), the following results were observed. For day 7, none of the treatment groups differed significantly from the control group or from one another. This nonsignificance is most likely due to the conservative nature of the adjusted P values when a large number of comparisons are being made. For day 14, all three treatment groups, AlloDerm alone, AlloDerm in platelet-poor plasma, and AlloDerm in platelet-rich plasma had significantly lower mean AI values than did the control group ($P < .0001$, $P < .0344$, and $P < .0002$, respectively). However, none of the three treatment groups differed significantly from one another. For day 21, both AlloDerm alone and AlloDerm in platelet-poor plasma groups had significantly lower mean AI values than did the control group ($P < .0001$ and $P < .0056$, respectively), although neither treatment group differed significantly from the other.

Angiogenic Index With Zeros

This analysis included wells that did not exhibit evidence of angiogenic blood vessel development (wells

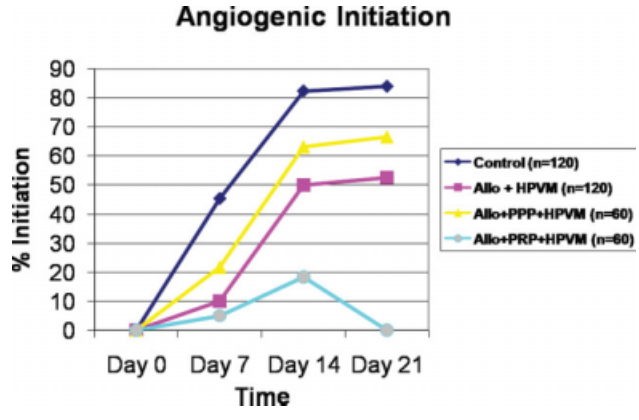

Fig. 2. Control (n = 120) versus AlloDerm (n = 120), AlloDerm plus platelet-poor plasma (PPP) (n = 60), and AlloDerm plus platelet-rich plasma (PRP) (n = 60) on human placental vein (HPVM) initiation. Placentas 1, 2, and 3. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE II.
Angiogenic Index Without Zeros.

Treatment Group Comparison	Difference in LS Means	Tukey-Kramer Adjusted *P* Value
Days in culture = 7		
Control vs. AlloDerm	1.29	.5308
Control vs. AlloDerm + PPP	2.15	.0944
Control vs. AlloDerm + PRP	0.76	.9983
AlloDerm vs. AlloDerm + PPP	0.86	.9949
AlloDerm vs. AlloDerm + PRP	−0.53	1.0000
AlloDerm + PPP vs. AlloDerm + PRP	−1.39	.9504
Days in culture = 14		
Control vs. AlloDerm	3.89	<.0001
Control vs. AlloDerm + PPP	2.22	.0344
Control vs. AlloDerm + PRP	3.75	.0002
AlloDerm vs. AlloDerm + PPP	−1.67	.4561
AlloDerm vs. AlloDerm + PRP	−0.14	1.0000
AlloDerm + PPP vs. AlloDerm + PRP	1.53	.7795
Days in culture = 21		
Control vs. AlloDerm	3.02	<.0001
Control vs. AlloDerm + PPP	2.72	.0056
AlloDerm vs. AlloDerm + PPP	−0.30	1.0000

LS = least-squares; PPP = platelet-poor plasma; PRP = platelet-rich plasma.

TABLE III.
Angiogenic Index With Zeros.

Treatment Group Comparison	Difference in LS Means	Tukey-Kramer Adjusted *P* Value
Days in culture = 7		
Control vs. AlloDerm	1.39	<.0001
Control vs. AlloDerm + PPP	0.90	.1463
Control vs. AlloDerm + PRP	1.47	<.0001
AlloDerm vs. AlloDerm + PPP	0.08	.6763
AlloDerm vs. AlloDerm + PRP	−0.49	1.0000
AlloDerm + PPP vs. AlloDerm + PRP	0.57	.3945
Days in culture = 14		
Control vs. AlloDerm	4.19	<.0001
Control vs. AlloDerm + PPP	3.53	<.0001
Control vs. AlloDerm + PRP	5.98	<.0001
AlloDerm vs. AlloDerm + PPP	−0.66	.9842
AlloDerm vs. AlloDerm + PRP	1.80	.0003
AlloDerm + PPP vs. AlloDerm + PRP	2.45	<.0001
Days in culture = 21		
Control vs. AlloDerm	4.52	<.0001
Control vs. AlloDerm + PPP	4.28	<.0001
AlloDerm vs. AlloDerm + PPP	−0.24	1.0000

LS = least-squares; PPP = platelet-poor plasma; PRP = platelet-rich plasma.

with a score of 0); hence, this measure can be viewed as representing a combination of a treatment's effect on both initiation and the subsequent growth of neovessels in this assay model (the AI). The analysis using a mixed-models approach revealed significant day (number of days in culture), treatment and interaction (day × treatment) effects ($P < .0001$). The tissue effect was not significant for this model ($P = .0545$). Because the day × treatment interaction effect was significant, post hoc F tests were conducted to check for significant treatment effects within each of the 3 measurement days. Results of the F tests revealed significant treatment effects for all 3 days, 7, 14, and 21 ($P < .0001$). Based on Tukey-Kramer adjusted P values for pair-wise comparisons among treatment groups within the 3 days (Table III), the following results were observed. For day 7, only the treatment groups AlloDerm alone and AlloDerm in platelet-rich plasma had significantly lower mean AI values than did the control group ($P < .0001$). Although the mean AI for the AlloDerm in platelet-poor plasma was lower than that of the control group, the difference did not reach statistical significance. Differences among the three treatment groups were not significant. For day 14, all three treatment groups, AlloDerm alone, AlloDerm in platelet-poor plasma, and AlloDerm in platelet-rich plasma had significantly lower mean AI values than did the control group ($P < .0001$). In addition, the AlloDerm in platelet-rich plasma had a significantly lower mean AI value than did either the AlloDerm alone or AlloDerm in platelet-poor plasma groups ($P = .0003$ and $P < .0001$, respectively). However, the difference between the AlloDerm alone and AlloDerm in platelet-poor plasma was not significant. For day 21, both AlloDerm alone and AlloDerm in platelet-poor plasma groups had significantly lower mean AI values than did the control group ($P < .0001$), although neither treatment group differed significantly from the other.

When placentas 2 and 3 containing all four groups were compared, significant increase in initiation was noted in the AlloDerm in platelet-poor plasma treatment group versus AlloDerm alone at day 21 ($P < .0001$). The differences in initiation between the control and AlloDerm in platelet-poor plasma, however, also reached significance at day 21 ($P < .0004$) (see Fig. 3) using Fisher exact test.

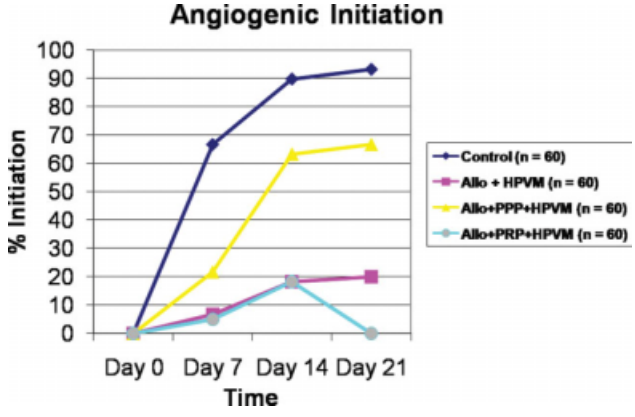

Fig. 3. Control (n = 60) versus AlloDerm (n = 60), AlloDerm platelet-poor plasma (PPP) (n = 60), AlloDerm platelet-rich plasma (PRP) (n = 60) on human placental vein (HPVM) initiation. Placentas 2 and 3 only. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Selected samples were compared histologically via hematoxylin and eosin staining and immunofluorescence to assess endothelial cell migration into the AlloDerm. In no instance were endothelial cells found within the AlloDerm. These endothelial cells tended to migrate around the periphery with limited penetration.

DISCUSSION

The use of extracellular matrices as a vehicle for delivery of growth factors to a compromised wound bed has been proposed as a method to enhance wound healing. AlloDerm has previously been shown in histologic studies to support fibroblast infiltration, neovascularization, and neoepithelialization in vivo.[1,18] In our study, AlloDerm in combination with placental vein discs was found to significantly reduce neovessel growth compared to control (vein discs only) groups. This finding may be related to the relatively decreased concentrations of matrix metalloproteinases in the fibrin-thrombin clot. In vivo, these enzymes are actively secreted by various cell types, including pericytes, endothelial cells, fibroblasts, and keratinocytes, whereas in our assay these are only secreted by endothelial cells. This may lead to a relative inhibition of migration of neovessels and human umbilical vein endothelial cells.

In vitro, AlloDerm limits the migration of human derived fibroblasts into the dermal matrix because of its dense collagen architecture and lack of pore-to-pore connectivity.[19] We observed that human placental vein endothelial cells also have limited migration into AlloDerm. Previous work has identified potential benefits of incorporating extracellular matrices into wound beds. Hodde studied the binding abilities of human microvascular endothelial cells (HMECs) to the fibronectin component of small intestinal submucosa (SIS), another ECM used for reconstructive purposes. He found HMECs readily attached to these matrices.[20] Hodde further suggests that the retention of growth factors, such as TGF-β and FGF-2 in SIS results in an ECM that possesses an inherent angiogenic capacity and incites blood vessel growth following implantation.[21] These angiogenic neoproliferative responses led other investigators to question the effects of bioengineered angiogenic tissues on tumor regrowth and metastases. In a study assessing the angiogenic response of a rat adenocarcinoma model, SIS and AlloDerm did not enhance tumor growth or metastases.[21] Additional mechanisms for inhibition of angiogenesis by AlloDerm in our model exist. The AlloDerm processing exposes the de-epidermalized dermis to rigorous detergents. This may be more important in smaller in vitro assays than in an in vivo situation. Longer rehydration times may be needed to dilute away chemicals potentially left behind.

Badylak et al. found early and aggressive angiogenesis when SIS was used as a scaffold in vivo, and that SIS could induce HMEC tube formation.[11,22] It has also been suggested by Badylak that ECM scaffolds can be considered as temporary controlled release vehicles for naturally occurring growth factors.[4] We studied the ability of AlloDerm with or without autologous blood products to act in this manner. The AlloDerm in platelet-poor plasma group revealed a significant increase in neovessel growth as compared to the AlloDerm alone group in placentas 2 and 3 ($n = 60$). The exact cause for this observation has yet to be elucidated, but potential mechanisms exist. The AlloDerm, as an adhesive matrix described above, may bind growth factors from the platelet-poor plasma and subsequently cause greater initiation than with AlloDerm alone. If this is true, however, why is it that the AlloDerm in the platelet-rich plasma group did not experience the same effect? Our group hypothesizes that the relatively higher concentration of platelets and white blood cells in platelet-rich plasma may be inhibitory to neovessel growth. A potential mechanism may include a graft versus host reaction as the platelet-rich plasma contained white blood cells and placental veins were derived from different donors, and the placental vein cells alone could be considered an immunocompromised host.

CONCLUSION

Based on our observations, we believe that AlloDerm significantly inhibits placental vein neovessel growth in this in vitro angiogenesis assay. Additionally, we demonstrated that AlloDerm can be enriched with platelet-poor plasma to stimulate angiogenic initiation. Further research is needed to validate this observation, assess its clinical relevance, and determine the clinical value of impregnation of AlloDerm with autologous blood products.

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